

What Is Claimed Is:

1. An isolated polynucleotide that encodes a bHLH-PAS polypeptide that is involved in binding juvenile hormone III.

2. An isolated polynucleotide as claimed in claim 1, wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:6.

3. An isolated polynucleotide as claimed in claim 1, wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide that encodes a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:5.

4. An isolated polynucleotide as claimed in claim 1, wherein said polynucleotide hybridizes under stringent conditions with a riboprobe that is the reverse transcript of a polynucleotide having the sequence of nucleotide 1514 through 1845 of SEQ ID NO:1.

5. An isolated polynucleotide as claimed in claim 1, wherein said polynucleotide hybridizes with a riboprobe that is the reverse transcript of a polynucleotide having the sequence of nucleotide 1514 through 1845 of SEQ ID NO:1, wherein said hybridization is carried out in 5X SSPE, 5X Denhardt's, 0.5% SDS, 50% formamide, and 100 µg/ml yeast tRNA for about 15 to about 17 hours at about 68°C.

6. An isolated polynucleotide of claim 1, wherein said insect is selected from the group consisting of

Coleoptera, Siphonoptera, Orthoptera, Thysanoptera,
Lepidoptera, Hemiptera, and Diptera.

7. An isolated polynucleotide of claim 1, wherein said insect is a member of the order *Diptera* selected from the group consisting of horn fly, fruit fly, screwworm fly, blow fly, mosquito, mediterranean fruit fly, biting midge, black fly, horse fly, deer fly, stable fly, leaf miner, housefly, bot fly, warble fly, tiger mosquito, swamp marsh mosquito, *Culex pipiens*, *Aedes aegypti*, and *Anopheles albopictus*.

8. An isolated polynucleotide of claim 7, wherein said polynucleotide has been isolated from a fruit fly.

9. An isolated polynucleotide of claim 8, wherein said polynucleotide has a nucleotide sequence that encodes a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:3 and SEQ ID NO:4.

10. An isolated polynucleotide of claim 9, wherein said polynucleotide has a nucleotide sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2.

11. An expression vector comprising the isolated polynucleotide of claim 1.

12. A cultured host cell comprising the expression vector of claim 11.

13. A host cell of claim 12, wherein said host cell is selected from the group consisting of bacterial cell, yeast cell, insect cell and mammalian cell.

16. The isolated polypeptide of claim 15, wherein said conservative amino acid variant is a polypeptide having an amino acid sequence that differs from the amino acid sequence of SEQ ID NO:3 by containing at least one amino acid substitution selected from the group consisting of (1) the substitution of an alkyl amino acid for an alkyl amino acid in SEQ ID NO:3, (2) the substitution of an aromatic amino acid for an aromatic amino acid in SEQ ID NO:3, (3) the substitution of a sulfur-containing amino acid for a sulfur-containing amino acid in SEQ ID NO:3, (4) the substitution of a hydroxy-containing amino acid for a hydroxy-containing

amino acid in SEQ ID NO:3, (5) the substitution of an acidic amino acid for an acidic amino acid in SEQ ID NO:3, (6) the substitution of a basic amino acid for a basic amino acid in SEQ ID NO:3, and (7) the substitution of a dibasic monocarboxylic amino acid for a dibasic monocarboxylic amino acid in SEQ ID NO:3.

17. The isolated polypeptide of claim 15, wherein said conservative amino acid variant is a polypeptide having an amino acid sequence that differs from the amino acid sequence of SEQ ID NO:4 by containing at least one amino acid substitution selected from the group consisting of (1) the substitution of an alkyl amino acid for an alkyl amino acid in SEQ ID NO:4, (2) the substitution of an aromatic amino acid for an aromatic amino acid in SEQ ID NO:4, (3) the substitution of a sulfur-containing amino acid for a sulfur-containing amino acid in SEQ ID NO:4, (4) the substitution of a hydroxy-containing amino acid for a hydroxy-containing amino acid in SEQ ID NO:4, (5) the substitution of an acidic amino acid for an acidic amino acid in SEQ ID NO:4, (6) the substitution of a basic amino acid for a basic amino acid in SEQ ID NO:4, and (7) the substitution of a dibasic monocarboxylic amino acid for a dibasic monocarboxylic amino acid in SEQ ID NO:4.

18. A method for screening compounds that specifically bind with a bHLH-PAS/JHR polypeptide, comprising:

- (a) incubating a test compound in a solution that comprises an isolated bHLH-PAS polypeptide, wherein said polypeptide is encoded by the polynucleotide of claim 1, and
- (b) detecting the binding of said test compound with said polypeptide.

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compound is detectably labeled.

step (b) using a scintillation proximity assay.

bioluminescent label.

2.

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25. The method of claim 24, wherein said detectably labeled juvenile hormone is [³H]10R-juvenile hormone III.

25. The method of claim 24, wherein said detectably labeled juvenile hormone is [³H]10R-juvenile hormone III.

27. The method of claim 18, further comprising the step of incubating said bHLH-PAS polypeptide with a detectably labeled photoaffinity analog of juvenile hormone after step (a) and before step (b).

27. The method of claim 18, further comprising the step of incubating said bHLH-PAS polypeptide with a detectably labeled photoaffinity analog of juvenile hormone after step (a) and before step (b).

- (a) a conservative amino acid variant of SEQ ID NO:3,
- (b) a functional fragment of a polypeptide having the amino acid sequence of SEQ ID NO:3,
- (c) a polypeptide having an amino acid sequence of SEQ ID NO:3,
- (d) a conservative amino acid variant of SEQ ID NO:4,
- (e) a functional fragment of a polypeptide having the amino acid sequence of SEQ ID NO:4,
- (f) a polypeptide having an amino acid sequence of SEQ ID NO:4, and
- (g) a Met-JHR alternatively-spliced isoform.

- (a) a conservative amino acid variant of SEQ ID NO:3,
- (b) a functional fragment of a polypeptide having the amino acid sequence of SEQ ID NO:3,
- (c) a polypeptide having an amino acid sequence of SEQ ID NO:3,
- (d) a conservative amino acid variant of SEQ ID NO:4,
- (e) a functional fragment of a polypeptide having the amino acid sequence of SEQ ID NO:4,
- (f) a polypeptide having an amino acid sequence of SEQ ID NO:4, and
- (g) a Met-JHR alternatively-spliced isoform.

29. A nucleic acid probe for detecting RFLPs in an insect population, wherein said RFLPs discriminate between JH-sensitive and JH-resistant individuals, said

probe comprising a genetic locus in a gene encoding a bHLH-PAS polypeptide that is associated with JH analog sensitivity and resistance traits, and wherein said polypeptide is involved in binding juvenile hormone III.

30. A method for detecting JH-resistant individuals in an insect population, said method comprising:

(a) obtaining a representative biological sample of said population; and

(b) detecting a nucleic acid sequence in said sample that corresponds to a predetermined sequence within a polynucleotide encoding a bHLH-PAS polypeptide that is altered in JH analog-resistant individuals, wherein said polypeptide is involved in binding juvenile hormone III.

31. A method according to claim 30, wherein said detecting step comprises:

(i) amplifying a nucleic acid sequence from said sample, wherein said sequence corresponds to a predetermined sequence within a polynucleotide encoding a bHLH-PAS/JHR polypeptide and wherein said sequence comprises at least one RFLP characteristic of JH analog resistance;

(ii) incubating said amplified nucleic acid with at least one predetermined restriction endonuclease, to form fragments;

(iii) size-separating said fragments to form a detectable pattern; and

(iv) comparing said pattern with a predetermined pattern obtained from JH analog-resistant individuals to detect the appearance of one or more RFLP characteristic of JH analog resistance.

32. An *in vivo* method for screening compounds that specifically bind with a bHLH-PAS polypeptide that is involved in binding juvenile hormone III, comprising:

(a) providing a host cell comprising (1) DNA encoding a fusion polypeptide comprising said bHLH-PAS polypeptide

and the DNA binding domain of a second polypeptide, (2) DNA encoding a heteromultimeric partner of said bHLH-PAS polypeptide and the activation domain of said second polypeptide, and (3) a reporter gene under the control of a minimal promoter driven by the response element for said second polypeptide;

(b) incubating a test compound with said host cell;
and

(c) detecting the binding of the test compound to said complex by monitoring expression of the reporter gene.

35. An *in vivo* method for screening compounds that specifically bind to a multimeric complex comprising a bHLH-PAS polypeptide that is involved in binding juvenile hormone III and the heteromultimeric partner of said polypeptide, comprising the steps of:

(a) providing a host cell comprising (1) DNA encoding a fusion polypeptide comprising bHLH-PAS polypeptide and the activation domain of a second polypeptide, (2) DNA encoding a heteromultimeric partner of said bHLH-PAS polypeptide and the DNA binding domain of said second polypeptide, and (3) a reporter gene under the control of a minimal promoter driven by the response element for said second polypeptide;

(b) incubating a test compound with said host cell;
and

(c) detecting the binding of the test compound to said complex by monitoring expression of the reporter gene.

36. An *in vivo* method for screening compounds that specifically bind with a bHLH-PAS polypeptide that is involved in binding juvenile hormone III, comprising:

(a) providing a host cell comprising (1) DNA encoding a fusion polypeptide comprising a bHLH-PAS polypeptide

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and the DNA binding region of a second polypeptide, (2) DNA encoding a bHLH-PAS polypeptide and the activation domain of said second polypeptide, and (3) a reporter gene under the control of a minimal promoter driven by the response element for said second polypeptide;

(b) incubating a test compound with said host cell; and

(c) detecting the binding of the test compound with said bHLH-PAS polypeptide by monitoring expression of the reporter gene.

37. A method according to any of claims 32, wherein said host cell is selected from the group of an insect cell, a yeast cell, and a mammalian cell.

38. A method according to any of claims 33, wherein said host cell is selected from the group of an insect cell, a yeast cell, and a mammalian cell.

39. A method according to any of claims 34, wherein said host cell is selected from the group of an insect cell, a yeast cell, and a mammalian cell.

40. A method according to any of claims 35, wherein said host cell is selected from the group of an insect cell, a yeast cell, and a mammalian cell.

41. A method according to any of claims 36, wherein said host cell is selected from the group of an insect cell, a yeast cell, and a mammalian cell.

42. An isolated polynucleotide which comprises the sequence of SEQ ID NO:6.

43. An isolated polynucleotide which comprises the sequence of SEQ ID NO:7.

44. An isolated polynucleotide which comprises the sequence of nucleotide 1 through nucleotide 1291 of SEQ ID NO:7.

45. An isolated polynucleotide which comprises the sequence of nucleotide 1 through nucleotide 1513 of SEQ ID NO:7.

46. An isolated polynucleotide which comprises the sequence of nucleotide 3733 through nucleotide 6235 of SEQ ID NO:7.

47. An isolated polynucleotide which comprises the sequence of nucleotide 4302 through nucleotide 6235 of SEQ ID NO:7.

(01 48. An isolated polynucleotide comprising the nucleotide sequence of the St-H fragment in vector pSt-H.

(01 49. The vector pSt-H.

50. An isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:7.

51. An isolated polynucleotide as claimed in claim 1, wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide having a nucleotide sequence of SEQ ID NO:7.